$$n(E < -\kappa^2) < \frac{6}{5} \int dr\, r|V_a(r) + 2V_b(r) + \kappa^2| \Big]_{V_a + 2V_b + \kappa^2 < 0}.$$

A somewhat more elaborate treatment follows from the remark that the matrix $V(r)$ defines three regions. In region I, $V_a < -4|V_b|$, and $-V$ is positive definite. Region II is characterized by $2|V_b| > V_a > -4|V_b|$, and here the matrix V is indefinite, while in region III, $V_a > 2|V_b|$ and V is positive definite. For a comparison potential, we use V itself in region I, the multiple $V_a + 2V_b$ of the unit matrix in region II, and zero in region III. There results the upper bound

$$n(E \leq -\kappa^2) < \int_{\mathrm{I}} dr[g_0(rr\kappa)|V_a(r)| + g_2(rr\kappa)|V_a(r) - 2V_b(r)|] + \int_{\mathrm{II}} dr(g_0(rr\kappa) + g_2(rr\kappa))|V_a(r) + 2V_b(r)|$$

and

$$n < \int_{\mathrm{I}} dr\, r\left[|V_a(r)| + \frac{1}{5}|V_a(r) - 2V_b(r)|\right] + \frac{6}{5}\int_{\mathrm{II}} dr\, r|V_a(r) + 2V_b(r)|.$$

Again, an alternative limit is obtained for $n(E \leq -\kappa^2)$ on replacing $V_a(r)$ with $V_a(r) + \kappa^2$ in the latter formula, with a corresponding redefinition of regions I and II.

In an application to a physical system, such as the deuteron, for which the distribution of energy values is known, these inequalities provide simple bounds on the potential used to represent the data.

* Supported in part by the Air Force Office of Scientific Research (ARDC).

[1] These PROCEEDINGS, **38**, 961 (1952).

[2] The physical constant $2m/\hbar^2$ is absorbed into the definitions of potential and energy.

[3] Some remarks in a very recent paper, L. Rosenberg and L. Spruch, *Phys. Rev.*, **120**, 474 (1960), footnote 21, indicate that these authors have considered similar questions.

A GLIA-NEURAL THEORY OF BRAIN FUNCTION

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> One day, I suppose, someone will find the clue and we shall then realize that we have been watching the missing mechanism at work in every experiment upon the brain that we did, but never recognized it for what it was.
>
> —B. Delisle Burns[1]

Theories of brain function abound, ranging from Aristotle's idea that it cools the blood to our present notion that its operations make behavior possible. Theories as to what these operations might be are not scarce either, and they extend from Descartes' idea (in which the pineal gland was supposed to move from left to right to permit humors to flow into one or the other of the brain ventricles) to the present-day almost universal view assigning to neurons alone the critical role. This neuron theory has generated much valuable information about brain function during the

past 75 years. The essay to follow suggests that a brain-model in which we visualize the glia and the neurons working together as the fundamental functional unit may provide an even more useful scheme for students of brain function to carry with them into their experiments.

Comparative anatomy demonstrates with reasonable clarity that every "brain," whether invertebrate or vertebrate, contains cells of two types—neurons and non-neurons. These latter we may for convenience call glia cells, bravely risking thereby the wrath of anatomists who would not include them all under that heading and disregarding the fact that many varieties of glia exist. A brain like our own contains vast systems of such glia cells, and they outnumber the neural elements perhaps tenfold. In electron microscope pictures, the area covered by these ubiquitous cells may exceed by a large factor that occupied by nerve cells and their processes. Hence glial cell cytoplasm takes up an astonishingly large fraction of brain volume, a fact never fully appreciated in the era of light microscopy with its cells badly shrunken by fixatives. An untutored observer constructing for himself a model of the brain from electron microscope pictures alone might well describe it as a huge collection of glia cells through which a nerve process occasionally wanders; those of us well instructed in the conventions, however, see a marvelously intricate arrangement of nerve cells held together by relatively unimportant non-neural elements. The point of view this essay urges is some golden mean between these two extremes, for this will be required if the reader is to evaluate the idea that glia cells might play an important role in the mechanisms responsible for learned and unlearned behavior. So much, then, for the anatomical facts upon which any useful model of brain function must solidly stand.

Naturalists, psychologists, physicians, and the man in the street know of many aspects of animal and human behavior for which a neural basis cannot now be provided. Let us consider merely one of these—hibernation in animals—as an example of the entire class. The bat, hamster, ground squirrel, and several other mammals periodically enter a cool place, drop body temperature, and go into what appears to be a deep sleep. In the laboratory, furthermore, the body temperature can be dropped well toward zero degrees centigrade, at which point neural activity in the brain also approaches zero and yet several complex adaptive responses persist.[2] When the animal wakens again, he proceeds as if nothing had happened to his nervous system at all, promptly exhibiting his normal repertoire of complex behavior, much of which must have been previously learned. Near-abolition of nervous activity thus fails at every point to destroy his most precious possession, knowledge of what to do to survive and to reproduce.

For many a professional neurophysiologist, a highly embarrassing fact of life is his failure thus far to deduce from the known properties of neurons any satisfactory conception of how this storage and retrieval of memories takes place. He has industriously assembled an impressive body of data pertaining to nerve excitation, conduction, and synaptic transmission, yet none of it relates very convincingly to such an obvious event as instant recall of a face once seen. Worse yet, the enormous repertoire of behavioral responses with which his new-born baby comes equipped—breathing, swallowing, crying, and sleeping, for instance—completely eludes his every effort.

The neurophysiological theorist today is wedded to the idea that neurons alone

regulate the responses that organisms emit. Yet, from what we know of neurons, they operate on a time base of milliseconds or seconds at the most, and the behavioral patterns handed down from one generation to the next or acquired as a habit and retained for a lifetime require some stable, utterly dependable storage system operating on a time base of hours, years, and generations. Millisecond neurophysiology, however arranged and rearranged thus far by its supporters, cannot clearly describe the mechanism of this storage to anyone's complete satisfaction. It is a curious fact that the glia cells, which could conceivably serve as that stable storage system, have apparently never seriously been assigned the chore. If, in any event, someone has developed the suggestion Nansen advanced in 1886[3] when he said neuroglia was "the seat of intelligence, as it increases in size from the lower to the higher forms of animal," his message has thus far had no impact whatever upon the main stream of western—or eastern—research on the brain.

In order to provoke some experiments, then, let me put Nansen's thought in strong modern words. The glia cells act in some unknown manner to organize neurons. They provide the basis for the "fields," "cell assemblies," and similar conceptions so many biologists—experimental and "theoretical"—have been forced to postulate. The electron microscope shows glia to invest, surround, and attach itself to nerve soma, axons, and dendrites—out to the finest terminals in the neuropil of C. Judson Herrick—and this may be so because that arrangement is precisely what enables neurons to transmit coherent, organized messages. Glia could receive afferent impulses, organize them somehow before permitting efferent outflow, and in still other ways yet to be discovered intervene so as to give order to neural events. A brain without glia would, in this conception, be a giant computer operating at random for lack of a program.

This notion is not to be confused with the far less comprehensive ideas expressed by Cajal and others who conceive the glia as a kind of insulator, especially at synapses. Nor do I mean that glia merely physically supports or metabolically nourishes neurons as has so often been suggested.[4] Perhaps glia does all these things, but in addition it would somehow "tell" the neuronal masses what they are supposed to do—in the same sense, I suppose, that the computer program "tells" its digital units what order and sequence of processes they must execute.

How, one may ask, could the glia cells become so wise? An answer buttressed by experiment does not come easily, but then neither does one that tells us how the undifferentiated cell develops during embryology into a liver or a gonad. Genetic mechanisms residing in glia cells could cause them to organize my neurons so that I breathe and remember just as genetic mechanisms actually keep my hair growing out brown in color year after year. This statement is not confirmed—nor is it contradicted—by any experimental fact I know.

When an embryo develops its first neurons, it also develops its first glia cells, and one can imagine that a mutual interaction between them, and a division of the labor they will always share, starts at that point in time. The remarkable findings of Weiss, Sperry, and others on regeneration of function in sense organs and motor apparatus[5] seem pertinent here. Their facts are simple: surgical operations on amphibia and fish can make chaos out of the normal neural connections (in brain and spinal cord) yet somehow fail to prevent normal function. For years, neither I nor anyone else has been able to understand how an eye, removed from its socket

and transferred to the opposite side, comes to act after its nerve regenerates as if it were still in its original site. But what if I suppose the glia of the retina or optic tectum "knows" where its neurons are supposed to be and how they are to act? The glia would then physically guide and physiologically control the neurons in that eye from beginning to end of the animal's life, and the surgical intervention of the experimenters merely provides another opportunity for that glia to do what its genes demand. When investigators report experiments proving Schwann (glia) cells "innervate" frog muscle—and give clear evidence of making functional connections with it—shortly after the peripheral nerve has been cut,[6] I can see an interesting clue to the way glia prosecutes its business. And when other investigators conclude from microelectrode studies that the frog's eye "speaks to the brain in a language already highly organized and interpreted instead, of transmitting some more or less accurate copy of the distribution of light on the receptors,"[7] this too becomes comprehensible, because according to the idea present here, glia would somehow organize the moment-by-moment activity of neurons as well as shepherd its flock of them mechanically and otherwise in embryology and regeneration. The experiments show regenerating optic fibers unquestionably do know exactly where to go and what to do in the brain and the authors insist that there is a "genetically determined representation of the world built into the tectum."[8] If I suppose the whole mysterious orderliness reduces to an expression of genetic properties of glia cells, there can be as much harmony and beauty here as when I suppose the neurons do it by themselves.

There is a host of other data, all well known, that strikes me as worth re-examining within this new conceptual framework. Many of us who record brain waves and the like find great difficulty getting the slow-wave (including DC) activity of the brain to emerge clearly out of mere nerve membrane-potential changes. In 1951, for instance, my colleagues and I were puzzled upon close examination of microelectrode records that an evoked slow wave could not possibly be a summation of nerve-cell electrical activity.[9] We did not then know what is now so clear from the electron microscope pictures—that a microelectrode tip must lie either inside neurons or inside glia cells and that there is no such thing as "extracellular recording" from brain and spinal cord. Suppose we hold the glia cells responsible for the slow waves and neurons for the spikes; here is a generalization that almost certainly cannot be exactly correct, but having boldly made it, we can now devise appropriate experiments to test it.

The latest fashion in brain research brings the electrical recording of brain waves to the problems of learning. An outstanding result is the discovery in the midbrain reticular formation of rhythmical activity that preserves the temporal features of the stimulus.[10] For instance, if you teach a cat that a light flashing seven times per sec means move or get shocked, you are likely to find a 7-per-sec brain wave present even in the dark. What brain structures give rise to this durable new brain wave? Conventional neurophysiological doctrine dictates that potential variation in many neuronal membranes is responsible, but I see no reason why an alternate theory in which a glia-neural complex of cells forms the functional unit cannot also be considered.

Now let us look at the cortical brain waves with which Berger's name is attached. They resemble in every respect—frequency spectrum, amplitude, variability, etc.—

waves recordable in gray and white matter anywhere else in the brain, including the new ones generated in midbrain during learning. A good many purely neuronal theories exist to deal with the cortical brain wave activity, many recent ones leaning heavily upon partial depolarizations in vertically oriented terminal dendrites. The only direct evidence that glia could help give the brain wave comes from Tasaki and Chang,[11] who showed that astrocytes produce an electrical wave, but the idea that glia might be involved, though suggested several times, seems not to have got very far.[12] Is it, however, a completely unreasonable thought? The neurons in all these areas from which brain waves are recorded vary enormously in size, distribution, number, and arrangement. How can the same electrical spectrum and amplitude possibly arise from axons in white matter and from nerve cells so diverse in the way synaptic buttons end upon them, in the detailed arrangement of their dendrites and axon terminals, and even in such physiological properties as complete or incomplete depolarizability? Perhaps that electrical activity displayed in common by such regions arises not so much from neurons as from those *other* cells that they share in common, the glia.

There is a phenomenon called spreading cortical depression with which neurophysiological theory has struggled for many years. In its presence, among other things, the animal cannot perform learned responses, the EEG is abolished, and a DC potential normally present between the ventricle and the cortical surface disappears. No comprehensive treatment of this constellation of facts has ever arisen from the neuron theory alone. If, however, we assume glia creates steady potentials and also displays electrical waves that signal its processes of organizing neurons, spreading depression, along with several other cortical electrical abnormalities, could conceivably be fitted into a realistic theoretical framework. Those who deal with human brain waves associated with sleep, brain tumors, epilepsy, and the like may find that this idea will illuminate their problems and lead to some experiments of value in their work.

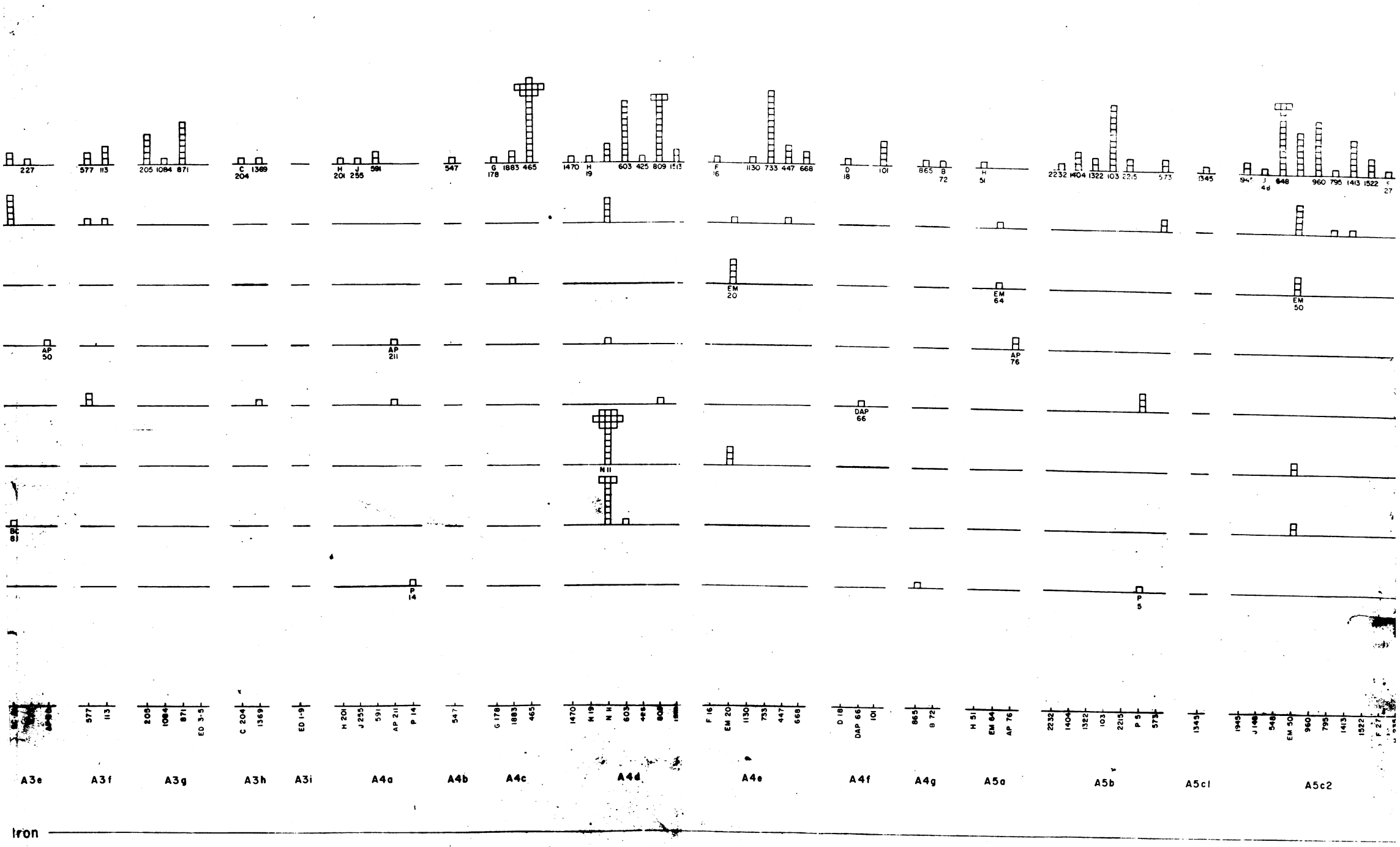
Let me continue this recital with two experiments so new they have not yet even been published. These are the straws that broke the camel's back, so to speak, and led to my writing this paper. This first was reported by Dr. Frank Morrell on October 20, 1960. With a microelectrode in the rabbit visual cortex he isolated a unit that discharged with a single burst of impulses to a flash of light. He then passed a weak current through the cortex while stimulating the eye with flashes at 5 per sec. Several minutes later, and with the current still flowing, he retested the animal with a single flash of light. The unit now responded not with a single burst but with a train of them at 5 per sec. As time passed, its tendency to give this complicated response to a single flash diminished, and after a half hour the single flash elicited the expected single burst. He thus elegantly demonstrated what actually had previously been shown,[13] namely, that a weak direct current reversibly modifies neuronal activity for a considerable period if the conditions are right. I am incapable of imagining how this phenomenon, which appears so closely related to learning, can be explained simply by supposing modifications of neuronal membranes. It is somehow much easier for me to imagine temporary alteration in the properties of the glia cells near to his electrodes. Either view, of course, is an entirely unsubstantiated guess, and an experimenter may take one or the other of them as the basis for his further investigations of the phenomenon.

On October 26, Dr. Gunnar Svaetichin reported the second experiment, a carefully controlled study of the electrical responses of a fish retina. He presented convincing evidence that one of three types of electrical response elicited by illumination appears only when the microelectrode lies inside a large horizontal cell, which is a glial cell in this retina. Another appeared only after the electrode had penetrated a Muller fiber, which Cajal himself identified as a glia cell. Both these forms of activity are sustained potential shifts, not spikes. Typical spikes can be recorded, of course, from the ganglion cells and optic nerve fibers.

It may be instructive now to consider how the glia might be involved in electroshock and in other brain stimulation studies, limiting consideration here to the so-called self-stimulation experiments that prove animals will go to a great deal of trouble in order to receive brain shocks.[14] That a monkey should press a key every few seconds for days, barely pausing for food and sleep, when the only result of this is to cause electrical shocks to be delivered deep inside his brain is certainly an observation to challenge any neurophysiological theory. Close examination shows neither the shock parameters (frequency, duration, and wave form) nor the exact brain area (within broad limits) to be highly critical factors. Shock intensity is, however, important, being linearly related over a reasonable range to the strength of the response according to recent as-yet unpublished data of Eliot S. Valenstein.

The prevailing view on how these shocks produce the brain events responsible for the compelling, complex, precise behavior invokes nerve-membrane depolarizations in the great limbic-midbrain neuronal system that recent research implicates in emotions, motivation, and the like. In short, the shocks stimulate nerve fibers and nerve cells. Many awkward assumptions are required, however, to deal with such matters as current spread, the loose requirements of stimulus parameters, the way neurons interact at synapses, etc. Let us however imagine the substrate upon which the shocks act to be primarily the great sheet of glia cells surrounding the soma and fiber tracts of the limbic-midbrain circuit. The shocks now would first activate this glia system (which incidentally would turn out to be much less finicky than neurons as far as shock parameters go), and the glia in turn would selectively and appropriately activate its neural compliment. The output of this joint effort looks like normal behavior because the shocks set off the same glia processes that occur in normal behavior. This construction placed upon the facts seems no more remote and unreasonable as a working basis than a hypothesis based upon the neuron doctrine alone.

What Karl Lashley might say of the idea that his engrams reduce to the genetic properties of glia cells would be interesting to know. He died baffled by the knowledge that no sensible relation exists between the location and extent of brain lesions on the one hand and the amount and kind of behavioral deficit these produce on the other. Superficial knife cuts that must utterly demolish intracortical neuronal organization make surprisingly little difference to behavior. Would he agree to the guess that this is so because the cortical glia cannot easily be damaged with a knife and that it restores itself after such a lesion, begins rearranging neurons, and does its best with whatever it can find? One gathers Heinrich Klüver might be favorably disposed to such a view, for in discussing the text of Lashley's final communication, he says:



5-bromouracil (*N*) *mutants:* These include the mutants of Benzer and Freese, which were induced by growth of T4B on *E. coli* B in synthetic medium containing sulfanilamide plus 5-bromouracil. Added to these are the data for mutants isolated in the presence of 5-bromodeoxyuridine and thymidine (Freese[5]). Dr. Freese contributed mutants representing sites not found in the first set.

5-bromodeoxycytidine (*BC*)*mutants:* The effectiveness of this mutagen on phage was discovered by Dr. J. Gregory, who kindly supplied a sample synthesized by Dr. D. W. Visser. The procedure used was the same as for the DAP mutants except that the mutagen was 5-bromodeoxycytidine at a concentration of 5×10^{-4} *M*. The average yield was 80 and the proportion of and mottled plaques was 0.8%.

Proflavine (*P*) *mutants:* These are the mutants, described by Brenner, Barnett, and Benzer, induced by proflavine during the growth of T4B on *E. coli* B. Each mutant was isolated from a independent burst.

TOPOGRAPHY OF THE GENETIC FINE STRUCTURE

FIG. 8.—*For detailed legend, unfold chart.*

PULL OUT CHART

(continued on reverse)

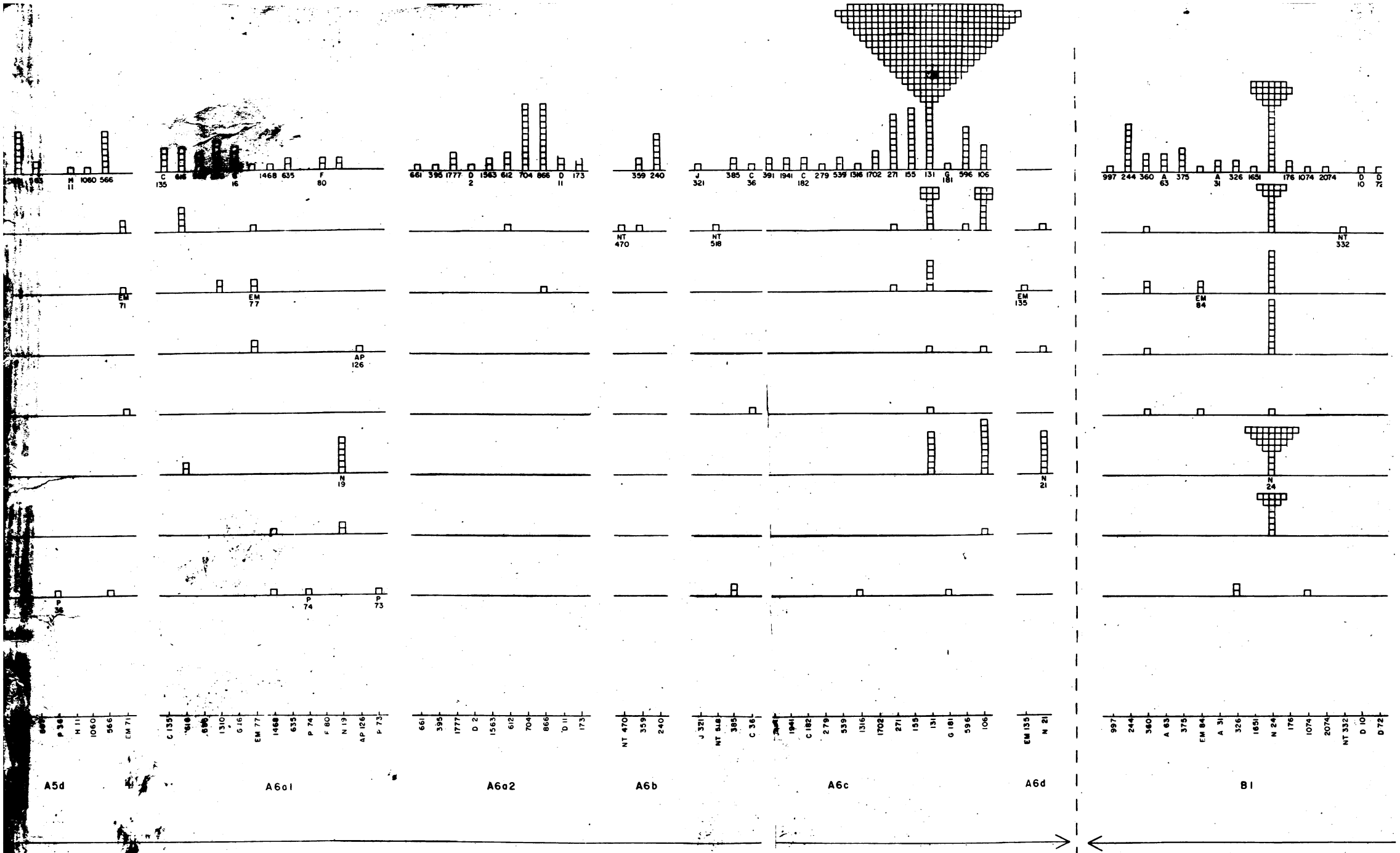


FIG. 8.—*continued. Full legend is given on front of chart.*

". . . it is of interest to recall that a famous physician Schleich, about 30 years ago wrote a book, *Vom Schaltwerk der Gedanken*, in which he tried to explain all psychological activities on the basis of neuroglial activities and in which he related thinking, remembering, forgetting, imagination, action, etc., to the contraction or relaxation of neuroglial processes. You may wonder whether Schleich's glial switchboard is really such a fantastic idea after you have seen Pomerat's tissue cultures containing cells with rhythmical pulsatile activity—cells which have been identified as oligodendroglial cells. The question may be raised whether psychoneurology—which at the present time seems to be chiefly 'psychoneuronology'—can afford to ignore the possibilities of a 'psychogliology,' so much the more since man, according to Friede, may be defined as the animal with the highest glial index."[15]

One additional thought needs development in conclusion. Desmedt and La-Grutta[16] report experiments showing that inhibitors of pseudocholinesterase, an enzyme localized in the glia, profoundly influence behavior, brain waves, and evoked response amplitude in the cat. This may be the first study clearly showing participation of glia in brain function, and its implications did not escape the investigators although they seem not to have expressed the central idea of this paper.

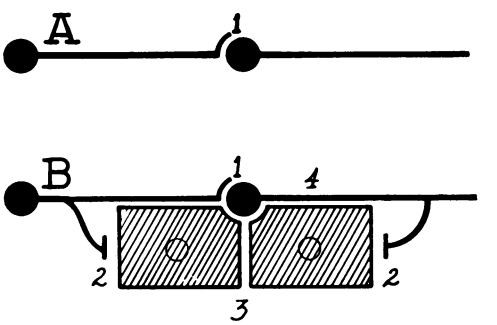


FIG. 1.—Diagrams showing elementary units of neural function according to present generally-accepted conventions (*A*) and the idea formulated here (*B*), which adds to synapses (1) what might be called gliapses, the neurono-glial (2), glia-glial (3), and glia-neural (4). All anatomical contacts between neurons and glia cells portrayed in *B* appear abundantly in electron microscope pictures of brain and spinal cord.

If this idea is correct in principle, glia must show exquisite sensitivity to a wide range of chemical substances and it doubtlessly manufactures them itself. One wonders therefore to what degree it is glia cells, not neurons, that react to excess hydrogen ion in the medullary respiratory center, drop out of action with barbiturate anesthetics, produce the depression of spinal reflexes in alcohol intoxication, and respond promptly to high blood levels of a sex hormone. Such chemicals do not influence all brain regions equally, and since there are many pharmacologically active substances, one must postulate in this scheme many classes of brain glia differentiated from one another on the basis of their biochemical specificities.

A brain map identifying glia-collections in terms of these biochemical specificities would be an intriguing new way to visualize and thus classify brain structures. Such a glia-anatomy would present the brain in the form of balls, sheets, rods, and complex 3-dimensional surfaces packed tightly together into a volume having the final external outline of the brain itself. To what extent the solid volumes outlined in such a glia-anatomy would coincide with the brain subdivisions conventionally taught in neuroanatomy is an interesting question. One might hope the coincidence would be exact, for otherwise medical students who already have such difficulty learning the way one great class of brain cells—neurons—are put together might rebel at having to learn an entirely different anatomical plan for the other—glia.

To summarize: This paper outlines a view of the brain diagrammed in Figure

1 in which its two cellular components—the neurons and the glia—mutually collaborate to produce behavior. Glia is here conceived as genetically charged to organize and program neuron activity so that the best interests of the organism will be served; the essential product of glia action is visualized to be what we call innate and acquired behavioral responses. In this scheme, neurons in large part merely execute the instructions glia give them.

This view arises as an inference from some old and modern data of anatomy, physiology, and the behavioral sciences. That powerful new anatomical tool, the electron microscope, shows glia to comprise a very large part of brain tissue; since no one knows what this glia mass does, one is at liberty to assign to it, conceptually, an essentially passive role, as is conventional, or a most highly active one, as is suggested here. Neurophysiology, dominated by the neuron theory of Cajal, has generated over the past 50 years a mountain of data without being able to formulate a convincing explanation for even such a commonplace behavioral event as remembering a name. Its data, furthermore, repeatedly imply that something else besides mere neuronal activity is at work. Could the "something else" needed to pull together disparate facts, harmonize apparent contradictions, and put an end to our journeys down blind alleys just be the physiological properties of that other cell population of the brain, the glia?

[1] Burns, B. D., *Can. J. Biochem. and Physiol.*, **34**, 380 (1956).

[2] Strumwasser, F., *Am. J. Physiol.*, **196**, 23 (1959).

[3] Glees, P., *Neuroglia, Morphology and Function* (Charles C Thomas: Springfield, 1955), footnote, p. 9.

[4] *Biology of Neuroglia*, ed. W. F. Windle (Charles C Thomas: Springfield, 1958). This book summarizes these theories adequately. The ideas of the Scheibels expressed at various places in the book will be of particular interest.

[5] Sperry, R. W., in *Handbook of Experimental Psychology*, ed. S. S. Stevens (New York: Wiley, 1951), chapter 7, pp. 236–280.

[6] Birks, R., B. Katz, and R. Miledy, *J. Physiol.*, **150**, 145 (1960).

[7] Lettvin, J. Y., H. R. Maturana, W. S. McCulloch, and W. H. Pitts, *Proc. of I.R.E.*, Nov., 1959, pp. 1940–1951.

[8] Maturana, H. R., J. Y. Lettvin, W. S. McCulloch, and W. H. Pitts, *J. Gen. Physiol.*, **43**, 129 (1960). The passage quoted appears on p. 164.

[9] Galambos, R., J. E. Rose, R. B. Bromiley, and J. R. Hughes, *J. Neurophysiol.*, **15**, 359 (1952). See pp. 373–5 in particular.

[10] Yoshii, N., P. Pruvot, and H. Gastaut, *EEG Clin. Neurophysiol.*, **9**, 595 (1957).

[11] Tasaki, I., and J. J. Chang, *Science*, **128**, 1209 (1958). See also W. Hild, J. J. Chang, and I. Tasaki, *Experientia*, **14**, 220 (1958).

[12] Windle, W. F., *op. cit.* See comments by Paul Glees, pp. 241–242.

[13] Rusinov, V. S., in *Russian communications of XX Internat. Cong. of Physiologists, Brussels*, **1956** (Moscow: Academy of Sciences of the USSR, 1956), pp. 346–349.

[14] Olds, J., and P. Milner, *J. Comp. Physiol. Psychol.*, **47**, 419 (1954).

[15] *The Brain and Human Behavior*, ARNMD Vol. 36, eds. H. C. Solomon, S. Cobb, and W. Penfield (Baltimore: Williams & Wilkins, 1958), p. 15.

[16] Desmedt, J. E., and G. LaGrutta, *J. Physiol.*, **136**, 20 (1957).